THE COMPARATIVE MORPHOLOGY OF THE WINTERACEAE
V. FOLIAR EPIDERMIS AND SCLERENCHYMA

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With three plates

FOLIAR CUTICLE

THE dried leaves of specimens of the Winteraceae frequently have a more or less conspicuously glaucous under surface, which fluctuates from faintly grayish to an intense uniform white. This glaucescence resolves under comparatively low magnifications into a number of distinct patterns. In most cases, the under surface of the leaf is speckled with white dots, *Figs. 1–6*. These dots vary in size and in number per unit area. They may be uniformly distributed and widely spaced, *Fig. 2*, or they may be aggregated and apparently coalesced in diverse patterns, *Fig. 3*. Furthermore, they may be surrounded by brownish tissue, *Figs. 1* and *2*, or they may be embedded in a grayish or white layer that coats the entire under surface of the leaf, *Figs. 4–6*. In extreme cases, e.g. leaves of certain collections of *Drimys granadensis* L. f., *D. brasiliensis* Miers, and *Pseudowintera axillaris* var. *colorata* (Raoul) A. C. Sm., the white layer may be so compact and thick that no spots are detectable within it.

The white spots are due to minutely granular or finely alveolar deposits in the oval or circular depressions in which the stomata are situated, *Figs. 7* and *8*. The alveolar substance covers the guard cells, *occluding the orifice*, and commonly extends outward over the adjacent subsidiary cells. In such leaves as those illustrated in *Figs. 1* and *2*, the finely alveolar material is localized over and about the stomata, whereas in those shown in *Figs. 4–6* it extends across the intervening areas, but in a thinner or less homogeneous form. Only in exceptional cases is the entire surface covered by a thick uniform layer of finely alveolar material which conceals the location of the stomata.

The white color is due to the presence of air in the interstices of the incrusting material. This may be demonstrated by dropping a glaucous leaf in boiling water. The leaf turns brown as the air is displaced by hot water, and the white color returns as the leaf is re-dried. This raises an important question regarding the extent to which the glaucescence of winteraceous leaves may be modified by differences in the drying or curing of herbarium specimens. The leaves of different collections of the same species frequently vary in color from brown to white. Not infrequently different leaves of the same collection or even of the same sheet exhibit similar variations in glaucescence. The conspicuously glaucous leaves of certain sheets exhibit more or less extensive brown discolored areas. A detailed microscopic examination of such discolored leaves indicates that

there was a migration and exudation of sap in the discolored tissue. As this sap evaporated, it left a brownish residue in the minute interstices of the incrusting material. The occurrence of browning during drying is dependent in part upon the structure and condition of the leaves and upon the thickness of the incrusting material.

We have found more or less conspicuous white stomatal areas on the leaves of most investigated species and varieties of all six genera of the Winteraceae. Thus the occlusion of stomata by deposits of minutely alveolar material appears to be an outstanding characteristic of the family. Where the stomatal plugs are not clearly discernible in ordinary surface views of the leaves, microscopic analyses of transverse sections demonstrate that they are concealed by papillae (*Drimys brasiliensis* pro parte), *Fig. 11*, by excessively thick layers of glaucescent material (e.g. certain collections of *D. granadensis* vars. *mexicana* (DC.) A. C. Sm. and *grandiflora* Hieron.), *Figs. 9* and *10*, or have been infiltrated with brownish residues during drying.

Since the publication of De Bary's (2) "Vergleichende Anatomie," granular, areolate, rod-like, and other types of structures on the outer surface of cuticles have commonly been referred to as wax or waxy coatings. It is significant in this connection, however, that although the incrusting material of the Winteraceae stains in Sudan III and is optically anisotropic, it does not melt in boiling water and is insoluble in boiling alcohol, hot ether, and other non-polar solvents. Thus, it exhibits none of the properties that are commonly assumed to be characteristic of plant waxes and, therefore, differs from the glaucescence of certain Magnoliaceae and Schisandraceae which is soluble in boiling alcohol and in ether at room temperature. The question arises, accordingly, whether the seemingly incrusting material of the Winteraceae is a distinct layer of different chemical composition or merely a physically different (i.e. more porous) outer part of the cuticle. The thick cuticles of the Winteraceae, as of many other plants, exhibit numerous intergradations between putative homogeneity and more or less conspicuously striated, lamellated, areolated, granular, ribbed, fluted, and warty structures. At present, there is no convincing evidence to suggest that any one of these diverse morphological forms is indicative necessarily of a waxy rather than of a cutinaceous composition.

As previously stated, the stomatal plugs of the Winteraceae usually have a uniform and finely alveolar structure, *Figs. 7* and *8*. They commonly grade off marginally (i.e. in the inter-stomatal areas) and more or less abruptly into varying admixtures of finely alveolar and coarsely granular or warty structures, which may grade in turn into more or less extensive and irregular patches of relatively homogeneous material. In certain cases, the entire under surface of the leaf may have a relatively thick coating of finely alveolar material upon which irregular masses of homogeneous material are superimposed, *Figs. 9* and *10*. The thick cuticle of the inter-stomatal areas is three-layered, *Fig. 10*, consisting of a homogeneous layer which grades into an alveolar layer which grades in turn into irregular

masses of homogeneous material. Since there are all intergradations of texture, it seems likely that the finely alveolar material of the Winteraceae may represent a physically porous phase of the chemically complex, cuticular emulsion. In many families, there obviously is a segregation of specific continuents (e.g. wax) of this complex emulsion upon the outer surface of the cuticle, but there are no *a priori* reasons for assuming that this must necessarily occur in the Winteraceae.

Our colleague, Dr. Smith (3), with whom we are collaborating in the study of woody ranalian families, has shown that the white stomatal areas are of some taxonomic significance in the classification of the Winteraceae. The consistent plugging of the stomata makes the family of interest from physiological and ecological points of view, and leads one to wonder whether there is any significant correlation between the peculiar stomatal and vascular structures within the family. The fact that there is no comparable plugging of the stomata in the vesselless *Tetracentron* and *Trochodendron* renders untenable any teleological inferences regarding the absence of vessels in the Winteraceae. The tendency toward reduction of scalariform pitting in the family might, however, be correlated with reduced transpiration through plugging of the stomata. It is of interest in this connection that the Coniferae (where scalariform bordered pits have been eliminated from both the metaxylem and the secondary xylem) are characterized by having stomata that are plugged with finely alveolar material.

PAPILLATE EPIDERMIS

The aerial organs of the Winteraceae with the notable exception of the carpels are characteristically glabrous. In certain cases, hairs are formed along the margins of bud scales and of young leaves, but the only tendency toward the formation of extensively distributed hairy structures on mature leaves is the papillate,. lower epidermis of *Drimys brasiliensis, Fig. 11.* Most specimens of the four varieties of this species, vars. *campestris* (St. Hil.) Miers, *retorta* (Miers) A. C. Sm., *angustifolia* (Miers) A. C. Sm., and *roraimensis* A. C. Sm., exhibit papillate surfaces, but certain collections of var. *campestris* (*Burchell 3567, Claussen 1064, Dusén 14504, Hoehne 1205* and *28700, Lützelberg 268*, and *Miers 4604*) do not. The papillae fluctuate considerably in form, length, and breadth, and in the character of their cuticular covering, which varies from finely alveolar to coarsely granular or warty. The absence of papillate surfaces in certain collections of *D. brasiliensis* var. *campestris* is not correlated with other significant morphological differences. Nor is the geographical distribution of these collections indicative of a stable glabrous variety of *D. brasiliensis*. As Dr. Smith (3) has shown, the morphological characters of the New World (*Wintera*) section of *Drimys* are relatively unstable. The various taxonomic entities are not sharply defined and may be differentiated only by their general trends of morphological specialization. Thus, the papillate character is variable and unstable in *D. brasiliensis* and by itself cannot be relied upon in differentiating taxonomic entities.

FOLIAR SCLERENCHYMA

The leaves of the Winteraceae fluctuate markedly in texture and thickness and exhibit corresponding variations in their internal structure. The cells of the epidermis and mesophyll vary in size, form, and arrangement, in the thickness of their walls, and in the character of their pitting. The cellular characters fluctuate so markedly within species and apparently also within different leaves of the same individual that it is difficult to utilize such characters in differentiating taxonomic entities without examining a much wider range of material than is available at present. There are, however, certain structures and certain trends of specialization in the leaves of the Winteraceae that deserve mention. The stomata of the Winteraceae are characterized by having from 2 to 6 subsidiary cells oriented parallel to the guard cells. The leaves of the family are also characterized by the presence of numerous spherical secretory cells such as occur in the cortex and pith of the stem and in the floral organs. Since both of these cellular characters are of common occurrence in woody ranalian families, they are not indicative of close relationship to any one of these families.

As indicated in the preceding paper (1) of this series, the larger foliar veins are jacketed by sclerenchyma in the *Wintera* section of *Drimys*, whereas the terminal veinlets are not, the only lignified elements being spirally or reticulately thickened tracheids, *Figs. 13* and *15*. The sclerenchymatous jackets of the larger veins are composed of slender, elongated, thick-walled cells. The leaves of this section of *Drimys* form, in addition, more or less numerous large, armed sclereids that are scattered through the spongy part of the mesophyll, *Figs. 13* and *15*. These sclereids are conspicuous features of the leaves of *D. brasiliensis* and *D. granadensis*, being poorly developed or absent in only a few collections of these species. On the contrary, they are absent or feebly developed in *D. Winteri* var. *andina* Reiche. They fluctuate in abundance in *D. confertifolia* Phil., *D. Winteri* var. *punctata* (Lam.) DC., and *D. Winteri* var. *chilensis* (DC.) A. Gray. In var. *punctata* of *D. Winteri*, they tend to be more numerous on either side of the midrib, whereas in var. *chilensis* they frequently tend to be unevenly thickened and to be associated with smaller more nearly isodiametric thick-walled cells.

In the *Tasmannia* section of *Drimys*, both the coarser veins and the terminal veinlets usually are embedded in more or less massive sclerenchymatous jackets, *Figs. 14* and *18*. Only infrequently does one encounter a specimen having terminal veinlets of the type which characterizes the *Wintera* section of the genus. In most cases, the veins have an inner jacket of elongated thick-walled cells and, in addition, an outer layer of shorter, broader lignified cells whose secondary walls fluctuate considerably in thickness in different specimens, *Figs. 14* and *18*. Interspersed sclereids, of the type which are formed so commonly in the *Wintera* section of *Drimys*, are of exceptional occurrence, having been encountered by us only in certain atypical specimens of *Drimys Brassii* A. C. Sm., *D. hatamensis* Becc., and

D. reticulata Diels. These interspersed sclereids are, however, conspicuously smaller and have less extensively projecting arms than those of the *Wintera* section. Such sclereids, of the type shown in *Fig. 17*, are of more frequent occurrence in *Pseudowintera axillaris* var. *colorata* (Raoul) A. C. Sm., but the veinlets of this plant, as of var. *typica* A. C. Sm., are somewhat intermediate in structure between those of the *Wintera* and the *Tasmannia* sections of *Drimys*.

In the six species of *Belliolum* available to us, the veins and terminal veinlets have sclerenchymatous jackets of the *Tasmannia* type. Isolated, lignified, thick-walled cells and clusters of sclereids are of sporadic occurrence in the leaves of *B. rivulare* v. Tiegh. (*Vieillard 2278*). The cells of the mesophyll in leaves of *B. crassifolium* (Baill.) v. Tiegh. (*Schlechter 15348*) have curious lignified reticulate thickenings, such as were encountered by van Tieghem (4) in the leaves of *B. Pancheri* (Baill.) v. Tiegh. and *B. Vieillardi* v. Tiegh. We have not observed such thickenings, however, in the leaves of *B. Burttianum* A. C. Sm. (*Kajewski 1680*), *B. gracile* A. C. Sm. (*Brass 2898*), *B. haplopus* (Burtt) A. C. Sm. (*Kajewski 1994*), *B. Kajewskii* A. C. Sm. (*Kajewski 2099*), and *B. rivulare* v. Tiegh. (*Vieillard 2278*).

In the leaves of the 17 species of *Bubbia* examined by us, the veins and terminal veinlets have sclerenchymatous jackets of the *Tasmannia* type, *Fig. 16*, but the mesophyll exhibits a wide range of structural variability. The cells of the mesophyll may have walls of relatively uniform thickness, *Fig. 16*, or they may have lignified reticulate thickenings as in certain collections of *Belliolum*. Isolated, interspersed, lignified, thick-walled cells, *Fig. 17*, which vary in form, abundance, distribution, and wall thickness, occur in more than half of the species examined by us. More or less massive clusters or nests of sclereids, *Fig. 12*, are formed in a number of species. In *B. pachyantha* A. C. Sm. (*Brass 4371*), they occur in association with interspersed, isolated sclereids; in *B. isoneura* v. Tiegh. (*Vieillard 17*), *B. semecarpoides* (F. v. Muell.) Burtt (*Kajewski 1216*), *B. sylvestris* A. C. Sm. (*Clemens 41142*), and *B. Whiteana* A. C. Sm. (*Kajewski 1495*), they occur among reticulately thickened mesophyll cells. It is of interest that, as shown in *Figs. 12* and *17*, the structure of the leaf may fluctuate markedly in different collections of the same species. In *B. sylvestris*, the walls of the mesophyll in *Clemens 41800* are of relatively uniform thickness throughout, whereas in *Clemens 4463* they are provided with reticulate thickenings, and in *Clemens 41142* with such thickenings in association with conspicuous nests of sclereids.

There is an even wider range of variability in the foliar structures of *Zygogynum*. Thus, the terminal veinlets of *Z. pomiferum* Baill. (*Balansa 2328*) and *Z. spathulatum* v. Tiegh. (*Vieillard 2266*) are without well developed sclerenchymatous jackets, the cells of the mesophyll have relatively uniform thickenings, and there are no interspersed sclereids or nests of sclereids. On the contrary, in *Z. Bailloni* v. Tiegh. (*Franc*), *Z. bicolor* v. Tiegh. (*Lécart 41*), and *Z. Vieillardi* Baill. (*Franc 1740*), the veins and

terminal veinlets are of the *Tasmannia* type, the cells of the mesophyll have reticulate types of thickenings, and both armed thick-walled cells and nests of sclereids are scattered through the mesophyll. The veins and veinlets are of the *Tasmannia* type in both species of *Exospermum, E. Lecarti* v. Tiegh. (*Lécart 144*) having a reticulately thickened mesophyll and *E. stipitatum* (Baill.) v. Tiegh. (*Vieillard 2281*) a thin-walled one with scattered more or less isodiametric sclereids.

The available evidence indicates that internal foliar characters are unstable and variable in the Winteraceae, particularly in the genera *Belliolum, Bubbia,* and *Zygogynum*. Much more extensive collections must be studied before attempting to utilize such characters as an aid in differentiating taxonomic entities. Sufficient material has been analyzed, however, to indicate that there are certain significant trends of foliar specialization in the Winteraceae. In the *Wintera* section of *Drimys,* increasing coriaceousness is attained largely by the formation of large, armed sclereids interspersed through the mesophyll, *Figs. 13* and *15*. On the contrary, in the *Tasmannia* section of *Drimys,* sclerification progresses along the veins and veinlets, the bulk of the mesophyll remaining thin-walled, *Figs. 14* and *18*. In *Belliolum, Bubbia,* and *Zygogynum,* increasing coriaceousness commonly involves intensified sclerification along the veins and veinlets, *Fig. 16,* the formation of interspersed sclereids and clusters of sclereids, *Figs. 12* and *17,* and not infrequently the formation of lignified thickenings throughout the mesophyll. In the more coriaceous species of *Bubbia* and *Zygogynum,* all three trends of sclerification may occur simultaneously.

As noted by van Tieghem (4), there are conspicuous variations in the occurrence and distribution of sclerenchymatous cells in the stems and petioles of the Winteraceae. In the stem, each vascular strand of the eustele is capped externally by slender thick-walled fibers and is subtended internally by elongated, lignified, thick-walled cells. During the earlier stages of the development of the secondary body, the external arcs of fibers may be fused into a more or less continuous ring of sclerenchyma by the sclerification of intervening parenchymatous elements. The later-formed secondary phloem is not stratified as in the Degeneriaceae, Magnoliaceae, and Annonaceae, but contains irregularly oriented patches of sclerenchymatous tissue. The pith and cortex may contain scattered sclerenchymatous cells of varied form, clusters of sclereids, or may be largely devoid of such structures. In general, there is a much more extensive sclerification of the cortex and pith in the Old World representatives of the Winteraceae. Particularly in the more coriaceous species of *Belliolum, Bubbia, Exospermum,* and *Zygogynum,* there tends to be an exaggerated development of clustered sclereids throughout the pith and cortex.

It should be mentioned in conclusion that crystal-bearing cells are of relatively infrequent occurrence in the lamina of winteraceous leaves. They have been observed by us only in *Bubbia Clemensiae* A. C. Sm. (*Clemens 4596*) and *Exospermum stipitatum* (Baill.) v. Tiegh. (*Vieillard 2281*). According to van Tieghem (4), there are no crystalliferous cells in the

stems and leaves of *Drimys*, but such cells occur in the stems of *Pseudowintera*, *Belliolum*, *Bubbia*, *Exospermum*, and *Zygogynum*, being commonly more or less closely associated with the medullary and cortical sclerenchyma.

LITERATURE CITED

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EXPLANATION OF PLATES

PLATE I

Lower surface of dry leaves photographed with reflected light. Magnification × 40.

FIG. 1. *Zygogynum Vieillardi* Baill., *Franc 1740*. FIG. 2. *Pseudowintera axillaris* var. *typica* A. C. Sm., *Kirk 347*. FIG. 3. *Bubbia longifolia* A. C. Sm., *Brass 13868*. FIG. 4. *Drimys piperita* Hook. f., *Elmer 9912*. FIG. 5. *Drimys Winteri* var. *chilensis* (DC.) A. Gray, *Hastings 355*. FIG. 6. *Drimys granadensis* var. *mexicana* (DC.) A. C. Sm., *Standley 42319*.

PLATE II

Figures 7–11: transverse sections of leaves stained with Haidenhain's haematoxylin and Sudan III and mounted in glycerin.

FIG. 7. *Zygogynum Bailloni* v. Tiegh., *Franc*. Showing occlusion of stoma by alveolar cutin, × 900. FIG. 8. *Drimys Winteri* var. *chilensis* (DC.) A. Gray, *Sargent*. Showing occlusion of stoma by alveolar cutin, × 1180. FIG. 9. *Drimys granadensis* var. *grandiflora* Hieron., *Holton 673*. Showing occlusion of stoma by internally alveolar and externally homogeneous cutin, × 900. FIG. 10. *Drimys granadensis* var. *grandiflora* Hieron., *Triana*. Showing 3-layered cuticle in interstomatal region, the middle layer being alveolar, × 900. FIG. 11. *Drimys brasiliensis* var. *campestris* (St. Hil.) Miers, *Mexia 5791*. Showing occluded stoma between two papillae, × 900. FIG. 12. *Bubbia Whiteana* A. C. Sm., *Kajewski 1495*. Cleared leaf showing clusters of sclereids, × 80.

PLATE III

Leaves cleared in hot dilute NaOH and mounted unstained in diaphane. Magnification × 145.

FIG. 13. *Drimys granadensis* var. *grandiflora* Hieron., *Cuatrecasas 6687*. FIG. 14. *Drimys rubiginosa* A. C. Sm., *Brass 12629*. FIG. 15. *Drimys granadensis* var. *grandiflora*, *Balls 5749*. FIG. 16. *Bubbia longifolia* A. C. Sm., *Brass 13868*. FIG. 17. *Bubbia Whiteana* A. C. Sm., *Brass 2278*. FIG. 18. *Drimys macrantha* A. C. Sm., *Brass 4519*.

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